Data Evaluation Record for a Non-Guideline Residue Study of Thiamethoxam (CGA293343) and its Metabolite Clothianidin (CGA322704) with A9807C Treated Winter Oil-Seed Rape Seed in Northern, France

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Guideline:	N/A
GLP Statements:	Yes
Test Formulation:	A9807C (280 g thiamethoxam L^{-1}, 8 g fludioxonil L^{-1} and 33.3 g metalxyl-M L^{-1})
Classification:	This study is classified as **SUPPLEMENTAL**. The residue data (thiamethoxam and CGA 322704) in winter oil-seed rape plants and honeybee products may be used quantitatively in risk assessments. The data for the visual assessments on brood development are of limited value because the thiamethoxam formulation included two other active ingredients. In addition, the exposure time (10 days after exposure) was short. The study deficiencies are summarized in page 5.
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Performing Laboratory:	Eurofins-GAB GmbH, Niefern-Öschelbronn, Germany Study Number: 2032746 Report Number: 20051041/F2-BZEU
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Summary

The purpose of the study was to determine the magnitude of residues of thiamethoxam (CGA293343) and its metabolite CGA322704 in crop and honeybee products, following use of a flowable concentrate mixture of thiamethoxam (280g thiamethoxam L^{-1}, 8g fludioxonil L^{-1} and 33.3g metalaxyl-M L^{-1} FS formulation referred to as A9807C) as a seed treatment for winter oil-seed rape. The study was carried out between April 25, 2005 and November 2006. In addition, the test and control beehives were visually inspected and the strength of the colony and presence of a healthy egg-laying queen were recorded to assess any adverse effects. The visual observation data are of limited value because the thiamethoxam formulation included two other active ingredients and the exposure time was inadequate. The residue data confirmed that the honeybees were exposed daily to ≤ 0.001(LOQ) ppm of thiamethoxam and metabolite CGA322704 when foraging on the winter oil-seed rape originated from the A9807C treated seed in the screened tunnels. The ranges of residues of thiamethoxam and CGA322704 for the crop and honey products were listed in **Table 1**.

Table 1. Summary of Thiamethoxam and Its Metabolite CGA322704 Residue Data

Matrix	Year	Control	Thiamethoxam (mg/Kg)	CGA322704 (mg/Kg)
Whole Plants	2005	<LOQ[1]	< 0.001	< 0.001 (LOQ)
Bee Pollen	2005	<LOQ	≤ 0.001	< 0.001 (LOQ)
Bee Nectar	2005	<LOQ	0.0006 – 0.0014	< 0.001 (LOQ)
Hive Wax	2005	<LOQ	< 0.0005 (LOQ)	< 0.001 (LOQ)
Hive Nectar	2005	<LOQ	< 0.0005 (LOQ)	< 0.001 (LOQ)
Hive Honey	2005	<LOQ	< 0.0005 (LOQ)	< 0.001 (LOQ)
Hive Pollen	2005	<LOQ	0.001	< 0.001 (LOQ)

[1]Limit of Quantitation (LOQ) for plants, soil, bee pollen and hive pollen is 0.0005 mg/Kg for thiamethoxam and 0.001 mg/Kg for CGA322704.

I. Study Location and Residue Sampling

Winter oil-seed rape seeds, pre-treated with A9807C or untreated as a control (only treated with fungicide thiram), were sown in Northern France on September 8, 2004. Honeybee colonies were maintained in mesh-covered tunnels and the bees were exposed to the flowering winter oil-seed rape in April, 2005. The samples of whole plants and honeybee products were collected at the trial site for residue analysis. The study defines DAY-1 as one day prior to the colony introduction into the mesh tunnels (April 25, 2005) and DAY0 as the first day (April 26, 2005) the hives were exposed to the thiamethoxam treated crop. The sampling schedule is summarized in **Table 2**.

Table 2. Sampling Schedule as Day after Exposure (DAY) for the Plants and Bee Products

	Year	Day after exposure (DAY) and Sample Intervals (S)[1]
Whole Plant, Bee Pollen and Nectar	2005	DAY2 (S1), DAY5 (S2), DAY9 (S3)

Comb (Hive Pollen, Honey and Wax)	2005	DAY-1(S1), DAY2 (S2), DAY5 (S3), DAY10 (S4), DAY22 (S5) DAY30 (S6), DAY60 (S7), DAY90 (S8), DAY120 (S9)

[1]The sample intervals (S1, S2, S3….) were corresponded to the DAE for each matrix and were used to track the samples from the field collection to the laboratory analysis.

II. Winter Oil-Seed Rape Seed Treatment and Application Rate

Treatment and drilling of the winter oil-seed rape crop and its maintenance until the set up of hives in the tunnels falls outside of this study report and is documented in a separate report (20041365/F1-BFEU –not submitted to the Agency). **Table 3** is an abbreviated summary of the available application information in this study.

Table 3. Summary of Thiamethoxam Concentration in Seed and Application Rate

	2005
Product application rate for A9807C	1.5 L / 100 kg seeds
Actual seed concentration (a.i. mg / kg seed)	4,200[1]
Actual seeding rate (kg seed / ha)	N/A[2]
Application rate (g a.i./ha)	N/A
Application rate (lb a.i./A)	N/A
Treatment (A9807C)	3 tunnels / 1 colony per tunnel
Control	1 tunnel / 1 colony per tunnel

[1] Calculated by the formula: 1.5 L/100 * 280 a.i. (g/L) *1000

[2] For details see final report 20041365/F1-BFEU

III. Test Bee Hives

The trial was conducted in Picardie, France. For the test, healthy colonies with young bees (1 queen and approximate 10,000 to 20,000 bees per colony) in hives with two boxes (lower box = brood chamber, and upper box = honey comb box) including 10 combs each were used. The colonies originated from one breeding line to guarantee uniform bee material. Each colony contained 6 – 7 brood combs with all brood stages and at least 14 – 16 combs with little honey or pollen stores to encourage the bees to collect pollen and nectar. The bees were free of symptoms of *Nosema* and other bee disease. The hives were introduced into the tunnels at the start of flowering of the winter oil-seed rape crop, in the morning before daily bee-flight had started on April 26, 2005 (DAY0). DAY0 was the first day that the honeybees were exposed to the treated winter oil-seed rape crop. The condition of the colonies was assessed prior to the introduction into the tunnels (DAY-1) in the evening and 11 days later (DAY10) at the end of flowering/honeybee exposure. The colonies were left in the tunnels for 10 days after set up. Thereafter, to minimize the further exposure to pesticides, the bee hives were relocated and maintained in the Wissembourg forest approximately 400 km away from the field site until mid-September.

IV. Brood Development Comparisons

In 2005, the test and control beehives were visually inspected and the strength of the colony and presence of the healthy egg-laying queen were recorded to assess any adverse effects after exposure to the A9807C formulation. It appears that there are no measureable effects on bee brood development between the A9807C treatment group (tunnels T1-T3) and the control group (tunnel C), or between the pre-exposure and end of the post exposure assessment. However, it may be premature to make a no observed adverse effect conclusion from the data because of the limited exposure and observation time (1 day pre-exposure and 10 days exposure time). The comparison of the brood development data is summarized in **Table 4**.

Table 4. Comparison of Brood Development in 2005

	T1	T2	T3	C
Pre-exposure assessment: April 25, 2005 (DAY-1)				
Strength (No. of combs covered with bees)	13.0	14.0	13.0	12.0
No. of combs with brood	7	6	6	7
Average area with eggs (%)	8.57	5.83	7.50	10.0
Average area with larvae (%)	9.29	15.83	11.67	9.29
Average area with pupae (capped cells)%	43.57	47.50	37.50	38.57
End of post-exposure assessment: May 6, 2005(DAY10)				
Strength (No. of combs covered with bees)	11.5	12.0	11.0	11.0
No. of combs with brood	7	7	6	7.0
Average area with eggs (%)	4.29	8.57	7.5	4.29
Average area with larvae (%)	5.71	5.71	5.0	2.14
Average area with pupae (capped cells)%	40.71	41.43	51.67	48.57

V. Sample Analysis

Aliquots of the samples were analyzed during March to October 2006. Hive product samples, (100 mg) were extracted by vigorous shaking with methanol : 0.2% formic acid in ultra-pure water (50:50 v/v). Aliquots equivalent to 50 mg were diluted with ultra-pure water. Sample clean-up was performed by solid-phase extraction (SPE) using Oasis HLB cartridges. Plant samples (unspecified amount) were extracted with methanol : water (50:50 v/v) and then the aliquots were diluted with ultra-pure water.

Thiamethoxam and its metabolite CGA322704 residues were determined by a high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) using matrix matched standards. The primary ions are 211.2 (m/z) for thiamethoxam and 169.0 (m/z) for CGA322704 respectively. The limit of quantification of the method was 0.0005 mg/Kg and 0.001 mg/Kg for thiamethoxam and CGA322704 respectively in hive honey, nectar, and wax and bee nectar. The limit of quantification of the method was 0.001 mg/Kg for both thiamethoxam and CGA322704 in hive pollen, bee pollen and plant samples.

In general, the method satisfies the repeatability criteria with acceptable mean recoveries (70-120%) and RSDs (≤20%). The linearity is established in the calibration (y=a+bx) using external standards for thiamethoxam (e.g. hive pollen: $r^2 = 0.9987$) and CGA322704 (e.g. hive pollen: $r^2 = 0.9957$).

VI. Study Limitations

The residue study is classified as **SUPPLEMENTAL.** The residue data may be used for quantitatively in risk assessments. The data for the visual assessments on brood development are of limited value because the thiamethoxam formulation included two other active ingredients and the short exposure time. The following are the major limitations:

1) The A9807C compound mixture of three active ingredients was used for the test. Thus, it is unknown if any effects (or lack of effects) were the result of thiamethoxam alone or a result of interactions among the active ingredients in the mixture.
2) The control has no replicate and has fungicide thiram for unknown reason.
3) Drilling of the winter oil-seed rape crop (= pesticide application information) and hive background data prior to the set up in the tunnels (20041365/F1-BFEU) are not provided to the Agency.
4) The condition of brood development between treatment and control groups was compared only for a short exposure time (10 days).
5) The limited hive numbers (3 treatment replicates and 1 control) and huge data variation limits the values of the bee data.
6) Data of bee mortality and abnormal foraging flight activity were not collected.
7) The residue samples were not analyzed immediately, but were stored for over 8 months at ≤ -18 °C. Since there were no matrix spike samples associated with the sample during sample collection and storage, the residue stability is uncertain. Therefore, it may be potentially underestimated the thiamethoxam and CGA322704 residue levels because of potential degradation during the sample storage.
8) The pesticide application history for the remote hive-relocation site is uncertain.
9) It is uncertain if the analytical method was validated by an independent laboratory.
10) The limit of detection (LOD) was not reported.
11) Calibration levels of standard concentrations were not provided
12) Images for mass spectrum were not included.
13) Secondary ions were not reported.